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Porous polymers and resins for biotechnological and biomedical applications

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Abstract

This review describes conventional and modern techniques of porous organic polymer synthesis. A huge variety of polymer architectures and functions can be gained by foaming, phase separation, imprinting or templating approaches. Several applications of porous polymers are discussed, focusing on biotechnological and biomedical applications, such as chromatography, protein synthesis, drug carrier systems, tissue engineering and others. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Macroreticular polymers; Supercritical carbon dioxide; Emulsions; Lyotropic phases; Cross-linking; Template synthesis

1. Introduction

Macroporous polymer gels, which are gels with pore sizes larger than those of homogeneously swollen polymer gels and below the size of polymer foams, were discovered in the 1950s. Since then, the procedures to make such gels have greatly improved, and the range of obtainable pore sizes, pore connectivities or morphologies, surface functionalities, and overall polarities have been increased. With growing versatility, new

promising fields of application have also been developed. Starting from the first applications of porous polymers as insulators and ion exchange resins, their outstanding performance in different types of column chromatography and as gel separation media was rapidly recognized. Meanwhile, porous polymers found applications as high tech materials for sensors, artificial antibodies or as enantioselective catalysts, and their use for electro-optical and (micro)electronic devices, implant materials and supports for flow-through reactors is currently being explored.

In contrast to inorganic porous gels, such as the well-known silica gel carriers, they offer some unique properties typical for the polymer scaffold, such as flexibility, ductility and the ability to in-

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corporate a very wide range of organic functional groups. In addition, they are comparably easy to make and — in case of the existence of larger industrial markets — comparably cheap.

This review discusses the general principles of porogenesis in polymers by different classes of pore-generating systems (porogenes) and the ability of surface functionalization. In the second part, a range of typical applications is discussed using selected examples from the literature.

2. Historical remarks

In 1935, Hermann Staudinger published the first systematic examinations of the copolymerization of styrene and divinylbenzene (DVB) in organic solvents (Staudinger and Husemann, 1935). It was found that the polymers become gel-like even at small DVB contents, and it was concluded that the new polymer had a three-dimensional network structure, with solvent-filled pores of the typical size of the distance between two cross-linking units, which are called in polymer science homogeneous gels. Such gels usually have pores in the range between 3 and 50 nm, and are rather soft when swollen with solvent.

Soon applications of such polymer gels were found, e.g. after sulfonation, as cation exchange resins. These gels, however, suffered from their mechanical flexibility and the insufficient access to the inner parts of the gel due to very small sizes of the pores and the non-hierarchical structure set-up. To improve the exchange properties, new processes were explored to generate macroporous poly(styrene-co-divinylbenzene) gels, the so-called macroreticular polymers. These polymers usually have high cross-linking densities (up to the point that they are made of pure cross-linker), they are mechanically more stable, and they provide larger pore superstructures which are also accessible for the polymer in non-solvents.

The basis of these new approaches discovered in the late 1950s, was the presence of porogenes in the polymerization which are removed from the polymer gel after polymerization. Examples of porogenes include structuring solvents, non-solvents or linear polymers (Seidl et al., 1967). In

the following years, the principle of using porogenes was expanded to a huge variety of processes for the synthesis of porous polymers using either liquid, gaseous or macromolecular porogenes. Within the last 10 years also, supercritical media (e.g. scCO_2) were added to the list of porogenes, which offers a number of structural and application advantages.

A modern approach towards porous polymers is the use of so-called templates. Although porogenes, like liquids and gases, are also called templates in a wider sense, we want to differentiate between porogenes which just create a pore by their geometrical presence, and templates which induce a special structure by specific interactions with the growing polymer matrix (e.g. electrostatic interactions, hydrogen bonding, or pattern recognition). Template synthesis concepts became increasingly important for supramolecular chemistry in the 1980s (Lehn, 1995), and they were employed from that time also for the generation of porous materials. Also the range of potential templates is spanning from single, functional molecules to colloid crystals and self-organized surfactant systems. By template synthesis, isotropic and anisotropic polymer morphologies with closed or opened pore structures can be obtained in a size range from the nanometer region to several micrometers.

3. Synthetic strategies to porous polymer networks

Most properties of porous polymers such as permeability, mechanical strength, transparency, size exclusion or adsorption capacity depend mainly on the topology or connectivity of the polymer strands or the pore system they constitute. A diversification of the range of obtainable porous polymer morphologies therefore became increasingly important, and each application is optimally served with a special architecture to be made (Table 1).

- Foaming techniques make use of gaseous porogenes. These might be generated by evaporation of solvents during temperature increase or pressure drop (e.g. polystyrene

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Table 1
Techniques of porous polymer synthesis

Method	Porogene	Pore sizes ^a	Morphologies	Examples of matrix polymers
<i>Foaming</i>	Gases Solvents Supercritical CO ₂	100 nm–1 mm	Isotropic, open/closed pores	Polystyrene, polylactide, polymethacrylate, poly(2- hydroxyethylmethacrylate), polyurethane, polycarbonate, etc.
<i>Phase separation</i> Thermally induced Chemically induced Immersion techniques	Solvents	1 µm–1 mm	Typically isotropic and open porous	Polystyrene, polylactide, poly(ether ether ketone), polyamide, polyepoxide, poly(dicyclopentadiene), etc.
<i>HIPE^b — polymerization</i>	Emulsions	10 µm–100 µm	Isotropic, open porous	Poly(styrene-co-divinylbenzene), poly(vinylbenzyl chloride-co- divinyl benzene), poly(aryl ether sulfone), etc.
<i>Template synthesis</i> Molecular imprinting Micellar imprinting Colloid crystal templating Polymerization of biocontinuous microemulsions Polymerization of liquid crystalline mesophases	Molecules Micelles Colloid crystals Bic. microemulsions Lyotropic mesophases PETs ^c	1 nm–10 nm 10 nm–50 nm 50 nm–1 µm 5 nm–10 µm 5 nm–10 µm	Isotropic, open/closed pores Isotropic/anisotropic open/closed pores	Poly(methacrylic acid), poly(ethylene glycol dimethacrylate), polyurethane, polystyrene, poly(hydroxyethylmethacrylate), Poly(acrylic acid), poly(acrylamide), poly(methyl methacrylate), etc.
<i>Other techniques</i> Mechanical stretching	Mechanical deformation	10 nm–100 nm	Anisotropic, open pores	Poly(propylene)
Interparticular cross-linking	Solvent	1 µm–10 µm	Isotropic, open pores	Poly(acrylic acid)

^aTypical pore diameters.

^bHIPE, high internal phase emulsion.

^cPET, polyelectrolyte–surfactant complexes.

foam), or are produced by chemical reactions during polymerization (e.g. polyaddition of urethanes). Because of the comparably high interface tension of gas bubbles in polymers, they undergo rapid Ostwald ripening, and the resulting pores can be open or closed, but are usually very large. Formation of finer pore systems relies on the addition of surfactants, e.g. silicone surfactants.

- The second concept of porogenesis in organic polymers relies on polymer-solvent phase separation processes. The phase separation can be induced throughout polymerization and cross-linking in different ways: (a) by addition

of a non-solvent to a polymer/solvent mixture (e.g. immersion techniques), chemically induced (e.g. the polymerization is performed in a monomer/non-solvent mixture, the polymerization itself depletes the monomer, and insolubility is induced), and (c) thermally induced phase separation (TIPS). For the latter three different mechanisms can be differentiated: physical gelation or crystallization, spinodal decomposition or combinations of both.

- Pores can also be implemented by the concepts of supramolecular and colloid chemistry, which aim for the generation of new function-

al materials by arranging matter on the nanometer to micrometer length scale. Syntheses within self-organized media are convenient one-step template reactions towards porous polymers and open manifold opportunities to control the pore architecture in a much broader range than possible by other techniques. The highest goal of a template synthesis, namely the direct replication of a self-organized structure into a permanent (polymeric) material, is, however, complicated by changes of the mixing thermodynamics throughout polymerization. In most cases partial kinetic control of the pore morphology and a coupled indirect structure control is achieved, only.

- In addition, there is a zoo of alternative synthetic or physical routes towards porous polymers and resins, such as interparticular cross-linking or mechanically induced pore formation, which, however, follow no common concept.

All these techniques will be discussed in the following sections in more detail.

3.1. Gases, liquids and supercritical CO_2 for pore generation

For the majority of polymer foams, i.e. porous polymers filled with a very high volume fraction of gas-filled pores, gaseous media are employed as porogenes. They are utilized in a wide range of applications, such as thermal and mechanical insulators, packaging materials, for medical devices and solid supports for catalysis. Volatile liquid, gaseous or supercritical media have been used as porogenes as they can be easily removed from the polymer matrix. The pore morphology depends on the type and amount of porogen and certain process parameters, such as degree of cross-linking, polymerization kinetics, extraction and drying methods.

Foamed polystyrene and related systems are produced by the addition of evaporable solvents (e.g. pentane) under high pressure. Pressure release and heating above glass transition temperature (T_g) results in foamed polymer particles. If

compressed or expanded in a mold, the well-known solid, foamed pieces are obtained. In some special cases, solid precursor salts can generate gaseous decomposition products. For the synthesis of highly open porous biodegradable poly(L-lactic acid) (PLLA) scaffolds for medical implants, ammonium bicarbonate was used as an efficient gas foaming agent as well as a solid salt porogen (Nam et al., 2000). For that, one injects a mixture of a solvent swollen PLLA and dispersed ammonium bicarbonate salt particles as a kind of paste in a mould. The mold is heated to induce the evolution of ammonia and carbon dioxide within the solidifying polymer matrix. This results in the expansion of pores, leading to well interconnected macroporous scaffolds having mean pore diameters of approximately 300–400 μm , which is an ideal size for high-density cell seeding.

Foams of poly(L-lactic acid) produced by a similar process based upon special hydrocarbons have been used to engineer a variety of different structures, including tissues with complex geometries such as implants for reshaping the human nose after serious accidents. The foams had densities as low as 0.12 g cm^{-3} , porosities as high as 87%, and specific surface areas of approximately $20 \text{ m}^2 \text{ g}^{-1}$ (Shastri et al., 2000).

The majority of porous polymers is, however, made by in-reaction precipitation from solvent-non-solvent mixtures, and liquid filled porous polymers (instead of foams) are obtained. In many cases, special hydrocarbon phases are employed for precipitation of the polymer and subsequent pore formation.

The porous polymer gels are essentially made in two different types of geometries:

1. As spherical gel particles or beads in the size range between 2 and 20 μm using suspension or stirring techniques. Such particles are then slurry-packed into stainless steel columns for size exclusion chromatography, HPLC, and other variations of chromatography. To optimize separation power at optimal flow, monodispersity of the particles is highly advantageous. The size of the particles seriously influences the pressure at which these

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columns have to be operated: the smaller the particles are, the higher the separation power, but the higher the pressure.

2. As monolithic objects, usually molded or cast in the final container to use, e.g. in glass capillaries, thin plates, or in stainless steel columns.

The results, with respect to porosity, are very different, and even exactly the same chemical system polymerized according to the same protocol at the same temperature can reveal very different pore architectures.

The preparation of larger macroporous polymer objects with hopefully homogeneous macropore structure occurs in an unstirred mold, and therefore has to be carefully controlled with respect to polymerization kinetics. Thermal heterogeneities or heating up due to insufficient transport of the polymerization will spoil all applications which rely on macroscopic gel homogeneity, e.g. all analytical applications. However, if carefully prepared, the larger macroporous objects obtained usually show extremely large channel systems, which provide the very high flow characteristic for monolithic columns and which will turn out to be advantageous for many applications in separation or catalysis. The only limitation is that the large channel system has to be homogeneous throughout the macroscopic body, a problem which can be handled by careful preparation.

In contrast, polymer beads prepared from identical polymerization mixtures, but in a suspension polymerization process, do not show these large flow-through channels, and only the macroporous system within the spheres is preserved. The main differences between the two processes are the lack of interfacial tension between aqueous and organic phases and the absence of shear forces resulting from stirring in the unstirred mould. Control of the kinetics of the overall process through changes in reaction time, temperature, and overall composition allows the fine tuning of the macroporous structure and provided an understanding of the mechanism of large pore formation within the monolithic porous gels. For example, a decrease in the reaction temperature,

that slows down the rate of polymerization, and the use of shorter reaction times than required for complete monomer conversion lead to porous objects with larger flow-through channels (Svec and Frechet, 1995b). This means that the large solvent 'highways' are essentially due to shrinking caused by an increased cross-linking density, which cannot be accommodated by reducing the outer dimensions, as in case of the spheres.

In spite of the formation of the very large pores, the monolith technique can nevertheless be expanded to rather large objects. The preparation of large cylindrical macroporous polymer monoliths with internal diameters of up to 50 mm has been studied for both styrenic and methacrylic monomer systems. The temperature profiles at different locations within the mold have been recorded during these polymerizations, and the occurrence of exotherms and temperature gradients was related to the creation of inhomogeneities in the pore structure. Several modifications, such as decreasing the rate of polymerization and a slow gradual addition of the polymerization mixture to the reaction vessel, were investigated in order to minimize the extent of the reaction exotherm. The latter approach proved to be especially powerful, as the absence of exotherms allowed the preparation of monoliths with homogeneous pore structures inaccessible by other methods for both monomer systems (Peters et al., 1997b).

3.1.1. Spheres

For the preparation of spheres, usually techniques of suspension polymerization are applied. Therefore the monomer/porogen mixture is dispersed in an inert solvent by use of special stabilizers. For hydrophobic monomers usually water is used as the dispersion medium, or high boiling hydrocarbons for polar monomers. It is obvious that the applied porogenes should not dissolve in or interfere with the continuous phase, that is they should have the same polarity as the monomer mixture.

A staged templated suspension polymerization method was used for the preparation of 5- μm beads from mixtures of styrene and several substituted styrene monomers, including 4-methyl-

styrene, 4-aminostyrene, 3-aminostyrene, 4-acetoxystyrene, and 4-*tert*-butoxycarbonyloxystyrene, with divinylbenzene in the presence of various amounts of linear polystyrene and dibutyl phthalate as porogenes (Lewandowski et al., 1998). The nature of the monomer as well as the total percentage of porogenic compounds in the polymerization mixture have a large effect on the porous properties and surface morphology of the monodisperse beads. Beads with large pores can only be obtained if the percentage of porogen in the mixture exceeds a threshold value to induce phase separation with the droplets sufficiently early in the network formation process. This threshold varies with the type of monomers involved in the polymerization. The level of incompatibility of the functional polymer gel strands formed by polymerization with the linear polystyrene that is present as an additional, structure controlling porogen in the polymerization mixture is another variable that affects both the pore properties and the outer morphology of the beads.

Another rather general example for the preparation of porous polymer particles is the precipitation polymerization of technical divinylbenzene (55 wt.%) in acetonitrile containing up to 40 vol.% toluene or other cosolvents. In this way monodisperse porous poly(divinylbenzene) microspheres can be obtained (Li and Stover, 1998). The prepared microspheres have diameters between 4 and 7 μm , total pore volumes of up to $0.52 \text{ cm}^3 \text{ g}^{-1}$, and surface areas of up to $800 \text{ m}^2 \text{ g}^{-1}$. As no surfactants nor stabilizers were used in the preparation of these particles, the generated particles are free of any residues of those molecules and can be employed directly. In the same publication, a typical procedure for evaluating the performance in a size exclusion chromatography application is described.

A new type of cross-linked, carboxyl telechelic microspheres (CTMs; telechelic as they contain endgroup-functionalized oligomeric chains) with different length of oligo-caprolactone chains, was prepared by a three-step procedure including: (1) the hydrolytic oligomerization of epsilon-caprolactone; (2) the esterification of oligo-caprolactone with maleic anhydride; and (3) the suspen-

sion polymerization of maleic acid polycaprolactone ester acid (MAPCLA) with divinylbenzene (Xie et al., 1998a). Toluene was used as a porogen. The CTMs, which are water swellable, spherical, and porous, contain carboxyl functionalities of $1.0\text{--}4.2 \text{ mEq g}^{-1}$ (dry). The morphology and porosity was tested by scanning electron microscopy and nitrogen adsorption. It turned out that the length of the telechelic chains and the degree of cross-linking (divinylbenzene), as well as the amount of porogen, are crucial for the exchange efficiencies of the CTMs.

3.1.2. Monolithic polymer gels

Polymer monoliths containing intricate pore networks can be obtained in any desired shape by the same simple molding process discussed above and provide unique advantages such as fast kinetics, high reactivity, and high throughput (Svec and Frechet, 1996).

The porosity and flow characteristics of macroporous polymer monoliths can be controlled during preparation by certain reaction parameters. Key variables such as temperature, composition of the pore-forming solvent mixture, and the amount of cross-linking divinyl monomer allow the tuning of average pore size within a broad range spanning orders of magnitude. The polymerization temperature, through its effects on the kinetics of polymerization, is particularly effective, allowing the preparation of macroporous polymers with different pore size distributions from a single composition of the polymerization mixture. The choice of pore-forming solvent is also important, larger pores being obtained in a poor solvent due to an earlier onset of phase separation. Increasing the proportion of the cross-linking agent present in the monomer mixture not only affects the composition of the final monoliths but also decreases their average pore size as a result of early formation of highly cross-linked globules with a reduced tendency to coalesce. The synergy of different effects was exemplarily examined using specific polymerization conditions and two monomer pairs, styrene–divinylbenzene and glycidyl methacrylate–ethylene dimethacrylate polymerized in closed molds (Viklund et al., 1996).

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In another study porous monolithic materials with high surface areas have been prepared from a technical grade 80 wt.% divinylbenzene (Xie et al., 1998b). The pore properties of these materials are again controlled by the type and composition of the porogenic solvent and by the percentage of cross-linking monomer (divinylbenzene) in the polymerization mixture. The surface area was found to increase with the divinylbenzene content of the reaction mixture. Using high-grade divinylbenzene and an optimized porogenic solvent, monolithic materials with specific surface areas as high as $400 \text{ m}^2 \text{ g}^{-1}$, yet still permeable to liquids at reasonable back pressure, were obtained. This material turned out to have an outstanding performance for solid-phase extraction, since phenols were extracted at flow velocities that exceed those of previous materials by a factor of 30. The incorporation of the polar monomer 2-hydroxyethyl methacrylate into the hydrophobic non-polar gel structure of the divinylbenzene monolithic material to improve wettability with water required the change of reaction conditions, in accordance with the altered miscibility behavior. With such a material, high recoveries of polar compounds are enabled while the high-flow properties of a monolithic material are maintained.

Photoinitiated free radical polymerization has been used for the preparation of porous polymer monoliths within UV transparent fused silica capillaries and quartz tubes (Yu et al., 2000). These formats were used as models for the direct preparation of the separation media within channels of microfabricated devices. A mixture of ethylene dimethacrylate, butyl methacrylate, and 2-acrylamido-2-methyl-1-propanesulfonic acid was polymerized in the presence of a porogenic solvent consisting of 1-propanol, 1,4-butanediol, and water at room temperature under UV irradiation. Modification of the porogen composition enables the tailoring of pore sizes within the broad range from approximately 100 to 4000 nm. Scanning electron micrographs confirmed the homogeneity of the porous structure of the materials prepared, even in a quartz tube with a diameter as large as 4 mm. Separation properties of the resulting capillary columns were tested in capillary electrochromatography (CEC) mode using mixtures

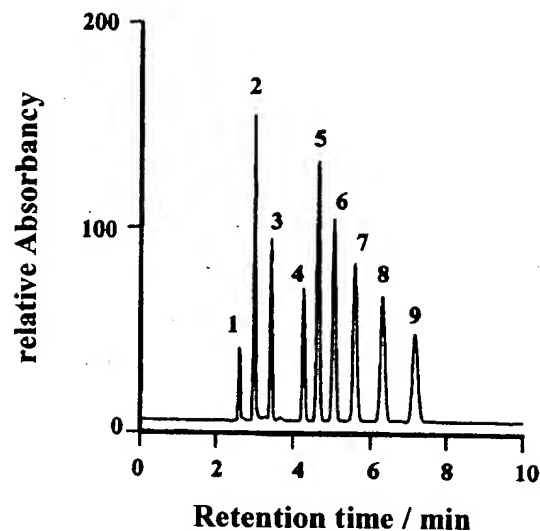


Fig. 1. Electrochromatographic separation of benzene derivatives using a monolithic, macroporous polymer. Peaks: thiourea (1), benzyl alcohol (2), benzaldehyde (3), benzene (4), toluene (5), ethylbenzene (6), propylbenzene (7), butylbenzene (8), amylbenzene (9) (Reproduced by permission from Peters et al. © 1998 American Chemical Society) (Peters et al., 1998).

of thiourea and eight aromatic compounds. The monolithic columns were also able to separate mixtures of peptides.

Rigid, monolithic capillary columns for reversed-phase electrochromatography have been prepared within the confinement of untreated fused-silica capillaries in a single step by a simple copolymerization of ethylene dimethacrylate, butyl methacrylate, and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) in the presence of a porogenic solvent (Peters et al., 1997a, 1998) (Fig. 1). The composition of the specifically designed ternary porogenic solvent made of water, 1-propanol and 1,4-butanediol allows fine control of the porous properties of the monolithic material over a broad range of sizes and compositions. The use of these novel macroporous monoliths eliminates the need for frits, the difficulties encountered with packed capillaries, and the surface functionalization of capillaries. Since the pore architecture and the composition of the monolithic materials can be easily tailored through changes in the composition of the ternary porogenic solvent, the effects of both pore size and the percentage of sulfonic acid monomer on the efficiency and the electroosmotic flow velocity

of the capillary columns could be independently studied over a broad range. A simple increase in the content of charged functionalities within the monolith leads to an expected acceleration of the flow velocity. However, increasing the pore size leads to a substantial deterioration of the efficiency of the separation. In contrast, monoliths with increasing levels of AMPS in which the pore size remains fixed due to adjustments in the composition of the porogenic solvent show no deterioration in efficiency while maintaining the same increase in flow velocity, thus producing a significant reduction in separation time. Since the electroosmotic flow through these capillary columns increases with both increasing pore size of the monolith and content of charged functionalities, better chromatographic properties have been observed for monoliths with larger surface area and a higher hydrophobicity. Using this technique, monolithic capillary columns with an efficiency higher than 120 000 plates m^{-1} have been obtained.

3.1.3. scCO_2 as a porogen

For the synthesis of porous polymers CO_2 is not only used as a gaseous foaming agent, but also, in the supercritical state, as a solvent. For instance highly porous sponges of biodegradable polymers are frequently utilized in tissue engineering both to transplant cells or growth factors, and to serve as a template for tissue regeneration. The classical processes used to fabricate sponges typically use organic solvents, either for expansion or plastization, but the organic residues remaining in the sponges usually harm the adherent cells, protein growth factors or nearby tissues. Polymerization and pore formation in supercritical CO_2 (scCO_2) enables the solvent free synthesis of porous polymers, which is of special interest for all biological and medical applications (Cooper, 2000), but also of general interest to avoid organic solvents in large scale production processes. Furthermore, scCO_2 shows the effect of lowering the T_g of many polymers. This effect can be used for the formation of microcellular foams by isothermal rapid decompression. During evaporation of CO_2 , T_g rises and reaching the foaming temperature the structure cannot grow

further and is locked in. For instance scCO_2 is a good swelling agent and plasticizer for poly(methyl methacrylate) (PMMA). Making use of this behavior, microcellular PMMA foams were made at constant temperature by a sudden pressure drop to induce phase separation in scCO_2 -swollen PMMA (Goel and Beckman, 1993a). The method is different from the usual temperature quench methods as it makes use of the isothermal glass transition depression in presence of scCO_2 rather than heating the polymer to above its normal glass transition temperature. Typically, the process leads to a microcellular core structure encased by non-porous skin. Average cell sizes (0.5–15 μm) and bulk densities (0.4–0.9 g cm^{-3} compared with 1.2 g cm^{-3} of the starting material) can be varied by changing the process conditions such as saturation pressure, temperature, cross-linking, and contacting time of the PMMA blocks at saturation pressure. The trends in observed cell density which increases very sharply with increasing pressure above 13.8 MPa (2000 psi), leveling out at approximately 27.6 MPa (4000 psi) (at 40 °C) are well described by classical nucleation theory. There is a corresponding decrease in the average cell size with increasing saturation pressure. The effect of temperature is rather small and gradual. The plasticization of poly(methyl methacrylate) (PMMA) networks by CO_2 as a function of pressure was also investigated by dielectric measurements (Goel and Beckman, 1993b).

On investigating the generation of microcellular polyurethane foam via reaction in carbon dioxide, it was observed that common polyurethane precursors, both isocyanates and polyols, are CO_2 -miscible at reasonably low pressures and temperatures. Usually fluorinated or siloxane derivatives are necessary to disperse the formed polymers in CO_2 . This new approach enables the formation of polyurethane foams from CO_2 , without any addition of stabilizers. By characterization of the phase behavior of several series of propylene oxide and ethylene oxide polyols, it was shown that the combined effects of molecular weight and hydroxyl number determine the phase separation pressures. In general, lower molecular weights and lower hydroxyl mole fractions shift the phase boundaries towards lower pressures. It



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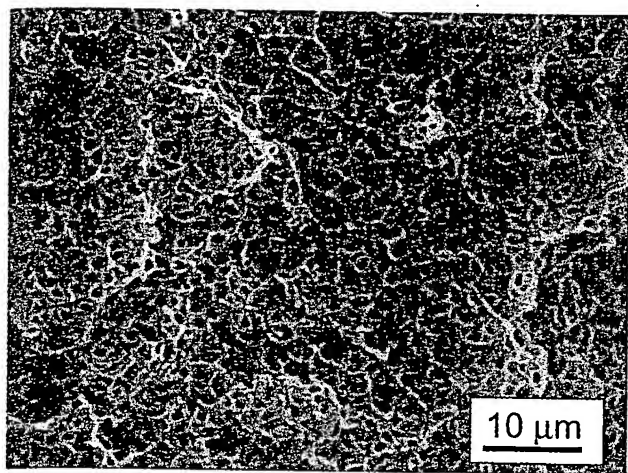


Fig. 2. SEM micrograph of a polystyrene foam. scCO_2 was used as a porogen (Reproduced by permission from Arora et al. © 1998 American Chemical Society) (Arora et al., 1998b).

also has been shown that CO_2 -soluble compounds may have a compatibilizing effect on other, less CO_2 -soluble, materials (Parks and Beckman, 1996a).

Also the foaming of polystyrene using supercritical CO_2 has been studied to better understand the microcellular foaming process, and for creating composite microcellular foams. Rapid decompression of CO_2 -saturated polystyrene at sufficiently high temperatures (above the depressed T_g) yields expanded microcellular foams (Fig. 2). The resulting foam structures can be controlled by manipulating processing conditions. Structures range from isotropic cells in samples retaining their initial geometry to highly expanded foams recovered in the shape of the foaming vessel and having oriented, anisotropic cells and limited density reduction. Higher saturation pressures lead to higher nucleation densities and hence smaller cells. Decreasing the rate of depressurization permits a longer period of cell growth and therefore larger cell sizes. Foams having a bimodal distribution of cell sizes can be created by reducing the pressure in two stages (Arora et al., 1998b).

In another study, microcellular polystyrene foams again prepared using supercritical CO_2 as the foaming agent were analyzed by mechanical measurements (Arora et al., 1998a). The cellular structures resulting from this process have been

shown to have a significant effect on the corresponding mechanical properties of the foams.

Foams of poly(ethylene terephthalate) (PET) and polycarbonate (PC) were prepared by rapid depressurization of CO_2 -saturated molten resins (Liang and Wang, 2000). It was found that the foaming temperature and pressure for PET and PC are nearly identical, and the attainable expansion ratio of the produced foam is generally less than 10.

Microcellular polyurethane foams can be generated using scCO_2 by a variety of techniques: through phase separation, which is induced by reaction-induced cross-linking, or by a pressure quench. The phase separation conditions are shown to impact the microstructure of the foams (Parks and Beckman, 1996b). Pore growth in such a system will occur through two mechanisms: by diffusion of CO_2 from polymer rich regions into the pores (reaction induced) and also through CO_2 gas expansion (expansion induced). Higher CO_2 pressures for polymerization (hence, higher fluid density) provide more CO_2 molecules for foaming, generate lower interfacial tension and viscosity in the polymer matrix, and thus produce higher cell densities. Increasing the functionality of the polyurethane precursors increases the T_g of the polymer network and leads to smaller cell diameters.

A method for the production of microporous poly(D,L-lactide-co-glycolide) foams which contain encapsulated proteins using supercritical carbon dioxide was described in another study (Hile et al., 2000). Aqueous protein emulsions in a polymer-methylenechloride solution were saturated with carbon dioxide at supercritical conditions, and then suddenly supersaturated by a pressure drop at ambient conditions causing bubble nucleation and precipitation of the polymer. Proteins contained in the water phase of the emulsion were encapsulated within the foams, including basic fibroblast growth factor (bFGF), an angiogenic factor of interest in tissue engineering applications. The release and activity of bFGF from these foams was determined in vitro and compared with similar porous scaffolds prepared by traditional solvent casting-salt leaching techniques. Total protein release rate was greater

from structures made in CO₂, than from those made by the salt leaching technique, but showed no primary release of burst bFGF, as it is typical for the salt leached structures. This provides a better use of the employed expensive factors, and active bFGF was released at a relatively constant rate. Residual methylene chloride levels were measured in the foams made with CO₂ implying that further solvent removal would be required prior to an in-vivo use.

Techniques to fabricate macroporous sponges from synthetic biodegradable polymers using high pressure carbon dioxide processing at room temperature are especially interesting for tissue engineering. Solid discs of poly (D,L-lactic-co-glycolic acid) were saturated with CO₂ by exposure to high pressure CO₂ gas (5.5 MPa) for 72 h at room temperature (Mooney et al., 1996). The solubility of the gas in the polymer was then rapidly decreased by a pressure drop to atmospheric levels. Nucleation and growth of gas cells within the polymer matrix resulted in this case in polymer sponges with large pores (approx. 100 µm) and porosities of up to 93%. Fiber-reinforced foams could also be produced by placing polymer fibers within the polymer matrix before CO₂ gas processing.

It must be underlined that employment of supercritical media allows gas bubble formation at minimal surface tensions or surface energies (close to the supercritical point), which according to the classical theories enables very rapid and homogeneous nucleation and a resulting very fine pore structure. In addition, a low surface tension also moderates the subsequent Ostwald ripening, that is the bubbles stay small for a longer time. These are very promising facts which might lead to the generation of nanofoams even without the addition of expensive and property disturbing surface active agents.

3.2. Porous polymers by thermally induced phase separation (TIPS)

Beside chemically induced phase separation and pressure induced separation of solvent and polymer phases, polymer-solvent phase separations can be induced by a temperature jump. In this

case generally three different scenarios can be distinguished: (1) thermally induced gelation/crystallization; (2) thermally induced spinodal decomposition; and (3) combinations of the first two mechanisms. In this section examples of all three mechanisms for the synthesis of porous organic polymers and resins will be discussed.

The microstructure formation in highly viscous polystyrene-cyclohexanol solutions after temperature jumps were studied by scanning electron microscopy and mercury intrusion porosimetry (Song and Torkelson, 1995). The polymer membrane microstructures can be tailored by controlling the quench route and coarsening time, as shown by a freeze-drying technique. Systems which are located in the phase diagram where they can undergo phase separation by spinodal decomposition show a well-interconnected, microporous structure with nearly uniform pore sizes, typical for the spinodal patterns known from the analysis of polymer blends. It was found that extending the phase separation time prior to freezing and solvent removal resulted in a significant increase in pore or cell size which is highly dependent on both quench depth and coarsening time. By using a two-step temperature process also complex microporous membrane structures with a bimodal pore size distribution were produced in this system.

Freeze-drying of polylactide solutions in 1,4-dioxane were studied as an alternative way to produce microcellular foams (Schugens et al., 1996b). The thermally induced phase separation was investigated in relation to several processing and formulation parameters. The effects of polymer concentration, chain stereoregularity, polymer molecular weight and cooling rate were examined in connection with the porous morphology and the mechanical characteristics of the final foams. As a rather general scheme, bundles of channels were formed with a diameter of approximately 100 µm. They had a preferential orientation that fits the cooling direction. A porous substructure with sizes of approximately 10 µm was observed in the internal walls of the tubular macropores. The porosity could be modified with respect to the pore architecture, density, solvent residue, mechanical resistance and degree of regularity in

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the spatial organization of pores by variation of the polymer concentration in 1,4-dioxane and polylactide stereoregularity. The cooling rate had a strong effect on the foam morphology, which is essentially controlled by the solvent crystallization. It turned out that in this case, the pores are created by a variation of the TIPS mechanism, where the pores are nothing but the copies of the 1,4-dioxane crystallites.

In another study thermally induced phase separation as a porogen technique has been investigated in an effort to produce a surgical implant suitable for cell transplantation (Schugens et al., 1996a). Here the liquid–liquid phase separation of solutions of amorphous poly D,L-lactide and semicrystalline poly L-lactide in a dioxane/water mixture was studied. The related temperature/composition phase diagrams had been set up by turbidimetry. By freeze-drying of some phase-separated polylactide solutions flexible and tough foams with an isotropic morphology were produced. Interconnected pores of 1–10 μm in diameter were expected to result from the spinodal decomposition of the polylactide solutions with formation of bicontinuous phases. The thermodynamics of the polymer/solvent pair had a decisive effect on the final macroporous foams, as shown by the dependence of their porosity, density, porous morphology, and mechanical behavior on molecular weight and crystallinity of polylactide and the polylactide concentration of the original solutions. On the basis of the foam characteristics, the potentials of the liquid–liquid phase separation (spinodal decomposition) had been compared with the solid/liquid phase separation (solvent crystallization) for pore generation.

Not only pure porous polymers, but also porous composites containing inorganic nanoparticles as functional factors have been synthesized. Hydroxyapatite mimics the natural bone mineral and has shown good bone-bonding properties (Zhang and Ma, 1999). The preparation and morphologies of three-dimensional porous composites from poly(L-lactic acid) (PLLA) or poly(D,L-lactic acid-co-glycolic acid) (PLCA) solution and hydroxyapatite (HAP) is regarded as a first step towards bio-reconstructable bone implant materials. A thermally induced phase separation technique was

used to create the highly porous composite scaffolds required for bone-tissue engineering. Freeze drying of the phase-separated polymer/HAP/solvent mixtures produced hard and tough foams with a bicontinuous structure of interconnected pores and a polymer/HAP composite skeleton. The microstructure of the pores and the walls was controlled by varying the polymer concentration, HAP content, quenching temperature, polymer, and the used solvent. The porosity increased with decreasing polymer concentration and HAP content. This way foams with porosities of up to 95% and pore sizes ranging from several micrometer to a few hundred micrometers were obtained, which are large enough to host bone cells. The composite foams also showed a significant improvement in mechanical properties over pure polymer foams.

Another example for the preparation of microporous membranes by TIPS is the thermally induced phase separation process of a ternary solution of HDPE, ditrydecylphthalate and hexadecane (Schugens et al., 1996a). Liquid–liquid phase separation was obtained even below the melting point by controlling the composition of the solvent mixture. It was shown that the membrane morphology can be controlled with different solvent compositions while the cooling conditions were kept constant.

By thermally induced phase separation also porous foams based on various biodegradable polymers of poly(L-lactic acid) and its copolymers with D-lactic acid and/or glycolic acid were produced (Nam and Park, 1999). Diverse foam morphologies were obtained by systematically changing several parameters involved in the TIPS process, such as polymer type and concentration, coarsening conditions and solvent/non-solvent composition. The produced foams had microcellular structures with average pore diameters ranging from 1 to 30 μm depending on the process parameters, as characterized by scanning electron microscopy (SEM) and mercury intrusion porosimetry. In an additional set of experiments, Pluronic F127 was used as an added surfactant to modify the pore geometry and size by lowering the interface tensions.

Meso- and macroporous membranes (pore sizes

~ 40 nm–1 μ m) of poly(ether ether ketone) (PEEK) were generated from solid–liquid phase separation of miscible polymer–polymer mixtures comprised of PEEK and polyetherimide (PEI), with subsequent extraction of the PEI diluent (Mehta et al., 1995). The introduction of a high polymer diluent provides a number of advantages in terms of melt processing and ultimate membrane properties. The nature of the solid–liquid phase separation and corresponding PEI segregation was investigated as a function of crystallization temperature and diluent molecular weight using dynamic mechanical thermal analysis. DMTA studies revealed segregation of PEI to both the interlamellar and interfibrillar/inter-spherulitic regions of the PEEK crystal superstructure. PEI extraction with liquid dichloromethane was found to be site specific, with the removal of the porogen polymer solely from the interfibrillar and interspherulitic regions; the retention of interlamellar PEI in the final porous polymer network was reflected in a significant increase in the thermal resistance characteristics of the material. It is underlined that PEEK and PEI belong to the so-called engineering plastics, i.e. the formed membranes are extremely resistant against temperature, mechanical stress, organic solvents, and other environmental wear and can be biotechnologically employed under incredible harsh conditions.

3.3. Porous polymers by immersion precipitation and chemically induced phase separation (CIPS)

Other techniques of porous polymer synthesis involving controlled phase separation are chemically induced phase separation and the technique of immersion precipitation. In this section some examples for both techniques are presented and compared to TIPS.

Porous polymers formed by immersion precipitation are synthesized by precipitation of a polymer solution in a non-solvent bath or a solvent/non-solvent mixture at constant temperature. Depending on precipitation conditions, such as composition of polymer solution and non-solvent bath, phase separation by nucleation and growth can be initiated for a liquid/liquid phase

separation process, a crystallization process or a combination of both processes.

An example is the formation of porous aliphatic polyamides (Nylon-6,6 and a Nylon-6, -6,6, -6,10 terpolymer) (Cheng et al., 1995a,b). Under certain conditions, crystallization of Nylon-6,6 results in a membrane with a uniform skinless microporous structure that is rapidly wetted by water. In contrast, liquid–liquid phase separation produces a polyamide film with largely unconnected cellular voids that is not wetted by water due to the different surface structures.

Anisotropic membranes were formed by immersion precipitation of a ternary polymer system consisting of bisphenol-A polycarbonate, *N*-methyl-2-pyrrolidone as solvent and water as non-solvent to the polymer (Di Luccio et al., 2000). The membrane morphology was characterized by SEM and related to some variables involved in membrane synthesis. Cloud-point measurements and precipitation kinetics, determined by means of light transmission experiments, showed that the studied system exhibits a very small miscibility gap, which favors instantaneous demixing by the mechanism of nucleation and growth. In unfortunate regions of the phase diagram (when directly immersed into a non-solvent bath), membranes with big macrovoids and low-pore interconnectivity were obtained.

Macroporous polyepoxides were synthesized by CIPS using bisphenol-A and 2,2'-bis-(4-aminocyclohexyl)-propane as precursors and hexane or cyclohexane as solvent (Kiefer et al., 1996). Depending on the hexane concentration both, monodisperse monomodal and bimodal pore size distributions were obtained. Pore sizes were all in the range of micrometers. The density of the porous polymer was significantly decreased without any loss in thermal stability compared to the neat matrix.

3.4. Polymerization of high internal phase emulsions (HIPE)

High internal phase emulsions (HIPEs) are dispersions of water in a continuous oil phase with internal phase volumes up to 99%, that is the water, although the majority phase, is not contin-

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ous. They form readily by adding water slowly to a stirred solution of a surfactant of low hydrophilic–lipophilic balance (HLB) dissolved in the oil phase. The internal or discontinuous phase has to be at least 74% of the emulsion volume, as this is the theoretical volume of a cubic close packing of spheres. In case that the continuous phase consists of, or at least contains, polymerizable monomers, HIPEs can be used for polymerizations to produce isotropic, open celled, polymeric foams (PolyHIPES) (Barby and Haq, 1982).

A range of polyHIPE polymers have been prepared by polymerization of the styrene–divinylbenzene continuous oil phase of a number of high internal phase emulsions (HIPE) (Hainey et al., 1991). The aqueous internal, or discontinuous, phase constituted approximately 90 vol.% in each case and consisted of small droplets of approximately 10 μm in diameter. Complete removal of the aqueous phase was achieved after polymerization by exhaustive extraction with hot ethanol, leaving monolithic polyHIPE polymers with completely interconnected cellular structures and an overall very low bulk density. By adjustment of the amount of cross-linker (divinylbenzene) and by use of either a precipitating porogen, petroleum ether, or a solvating porogen, toluene, in the comonomer oil phase, a secondary pore structure can be generated within the cell ‘walls’ of the polyHIPE polymers. Overall surface areas deduced from a BET treatment of N_2 sorption data of between 3 and 350 m^2/g have been achieved. Scanning and transmission electron micrographs have confirmed the cellular structure of the polyHIPE polymers, and the porous structure of their walls.

The formation of the interconnected morphology of open-cell styrene/divinylbenzene (DVB) polyHIPE copolymers has been studied by SEM on frozen HIPE samples at different stages of polymerization by cryo-SEM (Cameron et al., 1996a). The transition from discrete emulsion droplets to interconnected cells was observed to occur around the gel-point of the polymerizing system. This would suggest that the formation of holes between neighboring cells is due to the contraction of thin monomeric films on conversion of monomer to polymer, as a result of the

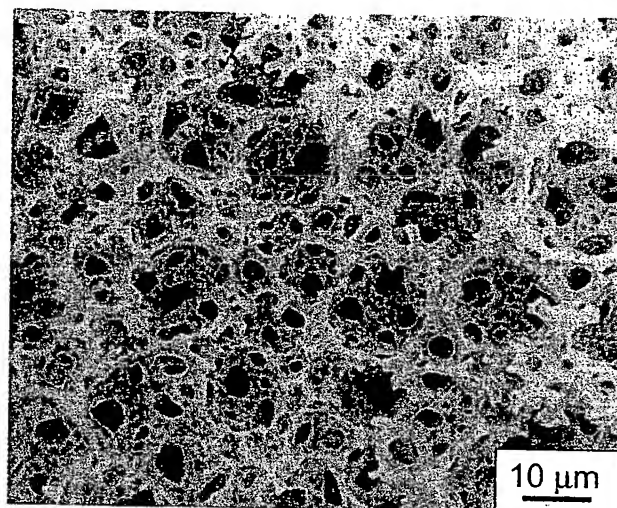


Fig. 3. EM micrograph of a poly(styrene-co-DVB) polyHIPE foam (Reproduced by permission from Cameron et al. © 1996 Springer-Verlag) (Cameron et al., 1996a).

entropic forces and conformational restrictions of the latter (Fig. 3).

Also the type of porogen added to the continuous phase of HIPEs containing divinylbenzene can strongly influence the morphology of the resulting foams (Cameron and Barbetta, 2000). The cell size was reduced as the solvent became a better cosurfactant, as inferred from surface pressure measurements of films representative of each HIPE continuous phase. In addition, this caused the windows connecting adjacent cells to increase, to such an extent that the cellular morphology was apparently lost and the porous gels were mechanically very weak. This could be compensated by an appropriate mixtures of the solvents investigated, producing in the optimized case materials with surface areas up to 554 $\text{m}^2 \text{g}^{-1}$, a cellular morphology and good mechanical properties.

Elastomeric and highly porous PolyHIPE materials had been prepared by polymerization of the continuous phase of high internal phase emulsions (HIPEs) containing styrene, divinylbenzene and varying amounts of either 2-ethylhexyl acrylate or the corresponding methacrylate monomer (Cameron and Sherrington, 1997).

Thermally stable polyHIPES were prepared from the engineering plastic material poly(aryl ether sulfone) (PES) (Cameron and Sherrington,

1997). For that, a maleimide-terminated aryl ether sulfone macromonomer was copolymerized with styrene, divinylbenzene (DVB), or a bis(vinyl ether) species in the continuous phase of a HIPE. Furthermore, a novel, non-aqueous HIPE methodology was employed, since only dipolar aprotic solvents were able to cosolubilize the polymeric precursor and surfactant. HYPES of petroleum ether, dispersed in a dipolar aprotic solution of maleimide-terminated PES, poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (PEO-PPO-PEO) block copolymer surfactants, comonomer, and 2,2'-azobisisobutyronitrile (AIBN), were used for polymerization. The cellular structures and porosities of the resulting materials were characterized by SEM, solvent imbibition, mercury porosimetry, and a BET evaluation of nitrogen adsorption results. All synthesized systems were shown to possess an open-cellular morphology and a secondary pore structure within the polymer walls. Thermogravimetric analysis (TGA) of the materials indicated that critical degradation occurred at higher temperatures than in poly(styrene/DVB) polyHIPE but lower than the PES macromonomer precursor.

3.5. Porous polymers and resins by template syntheses

The most general definition of a template is one with a structure directing agent. In case of direct or 'true' templating, the templated material is a 1:1 copy of the template structure. During synthesis no changes in order and scale length of the template structure occur, and in the present case, the resulting polymer contains pores with the shape and size of the porogen. In difference to direct templating, indirect templating results in a metamorphic reconstruction, e.g. by molecular rearrangements during the synthesis. Due to the pre-organization in the presence of the template, the directed rearrangement or phase separation often leads to highly ordered materials, which, however, contain the template in a rearranged morphology. Therefore, the structural organization of organic materials by template synthesis

shows many similarities to the generation of complexity in biological matter, where three different scenarios of morphogenesis are to be distinguished (Mann et al., 1997):

- the polymerization within a template structure preserving the structure, which results in a replica of the template (transcriptive synthesis);
- the interplay of the polymerization process with the (usually) self-assembled template results in a modified common/template structure which, however, is well defined with respect to thermodynamic arguments (synergistic synthesis); or
- reactions which take place in spatially restricted reaction fields directed by morphological reconstruction (reconstructive synthesis). In this latter case often complex morphologies and also hierarchical structures are obtained, usually ordered on a larger length scale than the template structure.

Which of these scenarios are realized for a specific template synthesis depends mainly on thermodynamic factors. The compatibility of the formed polymer matrix with the template structure is — among other effects — strongly related to attractive or repulsive interactions at the interface. Matching the electrostatic charge-density or controlling hydrogen bonding are important for enthalpic interactions. Furthermore, entropic interactions play a key role, e.g. the restrictions of polymer chain conformations in a spatially confined reaction field. In this context the copolymerization of hydrophobic with hydrophilic or amphiphilic monomers and the variation of cross-linking density are important tuning parameters for structure control.

Beside the templating by molecules, colloid crystals and self-organized media, also macroscopic objects with an internal microstructure can serve efficiently as templates.

In the following, the different techniques of templating by molecules, colloidal crystals, self-assembled (surfactant) structures, and microscopic objects will be separately discussed.

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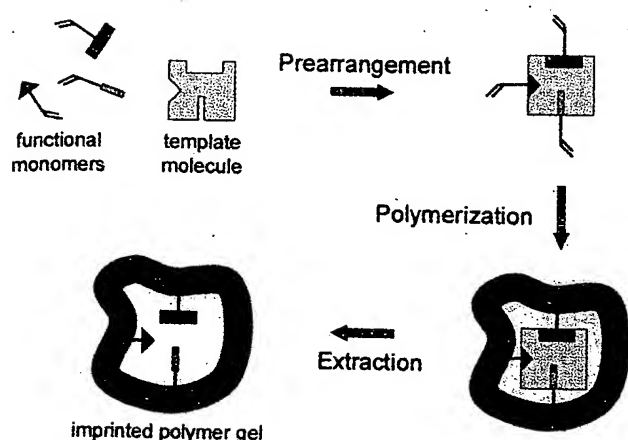


Fig. 4. Schematic representation of the molecular imprinting process.

3.5.1. Molecular imprinting

Molecular imprinting is a technique for template synthesis of polymers with pores structured on a molecular scale (Steinke et al., 1995; Wulff, 1995). This way synthetic polymers gels be designed where pore and molecule complement each other, and highly specific recognition sites with binding capabilities similar to those found in biological systems are to be expected (e.g. enzymatic catalysis). The synthesis is usually performed by copolymerization of functional and cross-linking monomers in presence of a molecular template (imprint molecule) and additional solvents as porogenes. The functional monomers have to interact with the template molecules by covalent or non-covalent bonding. After polymerization the template molecules are removed by extraction or chemical cleavage leaving molecular imprinted cavities according to the shape and chemical structure of the imprint molecules (Fig. 4). Recently several reviews were published discussing the synthesis of molecular imprinted polymers (MIPs) and also their application in separation (Ramstrom and Ansell, 1998; Sellergren, 2000; Takeuchi and Haginaka, 1999; Vallano and Remcho, 2000) and sensing (Yano and Karube, 1999).

For most molecular imprinting approaches smaller molecules are used as templates, such as amino acids (Lin et al., 1998), sugars (Mayes et al., 1994), oligo-peptides (Klein et al., 1999) or

steroids (Cheong et al., 1998). The obtained porous polymers carry a functional and stereochemical memory like artificial antibodies (Takeuchi and Matsui, 1996) or enzymes and can be used as stereo- and regio-selective solid supports. Potential applications are separation media in liquid chromatography, solid-phase extraction, capillary electrophoresis and membranes (Heegaard et al., 1998; Schweitz et al., 1998; Witsuma and Schurig, 2000). Molecular imprinted polymers are not only used for analysis and separation techniques but also for synthesis, e.g. in enantioselective catalysis (Locatelli et al., 1998). Also examples for artificial antibodies and immuno-assays are given in literature (Haupt and Mosbach, 1998).

The most successful MIPs use non-covalent binding and functional monomers which are able to interact with the imprint molecule via hydrogen bonding and electrostatic interactions, such as methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA). A distinct flexibility of the polymer is to make the imprinted cavities flexible and accessible. On the other hand a too high flexibility of the polymer matrix leads to a loss of structural definition. Other limiting factors are a too low or too high polarity of the template molecules. In the first case the interactions to the functional monomers are not specific enough, in the latter the solubility in the monomer solution is usually too low. Also, too big sizes of imprint molecules can be a limiting factor. Some reports give examples for successful templating of bigger molecules like proteins or even cells but often with limited recognition capabilities. A promising approach towards templating bigger entities like proteins is the use of additional mediators for optimization of the polymer — template assembly, e.g. by surface coating with disaccharides (Shi et al., 1999).

Mimicking the hydrolytic activity of chymotrypsin was realized by grafting Co(II) coordinated monomer-template assemblies of different monomers (such as *N*-methacryloyl-L-serine, *N*-methacryloyl-L-aspartic acid and *N*-methacryloyl-L-histidine, using *N*-nicotinoyltyrosylbenzyl ester as the template) on a porous support made of

poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate) [poly(GMA-EGDMA)]. The functionalized porous supports were found to be hydrolytically active against the 'chymotrypsin specific' substrate *N*-benzyloxycarbonyl tyrosyl-*p*-nitro phenyl ester. The cooperative effect among the functional groups was demonstrated by comparing the hydrolytic activity of the mimic with other polymers comprising only imidazole groups or hydroxyl and imidazole groups. The observed kinetics for the hydrolysis of substrate *N*-benzyloxycarbonyl-tyrosyl-*p*-nitrophenyl ester obeyed Michaelis–Menten kinetics. The effect of molecular imprinting of the template molecule in enhancing the substrate specificity of the mimic was demonstrated. The mimic synthesized in the absence of Co(II) was found to be hydrolytically inactive (Lele et al., 1999b). For a series of mimics containing the same functional monomers, the surface area of the support decreased with increased levels of grafting, which led to both higher k_{cat} and K_M (k_{cat} : catalytic constant, indicator for enzymatic activity). These trends are consistent with those reported for reactions using immobilized enzymes (Lele et al., 1999a).

Not only organic molecules but also inorganic assemblies such as calcite crystals can be used as templates for MIP synthesis. Such polymers show specific capabilities to direct crystal growth of unusual modifications, e.g. the growth of calcite crystals under conditions where usually aragonite would be formed (D'Souza et al., 1999).

Highly specific recognition is the prior condition for many potential applications of molecular imprinted polymers, such as sensors, waste water purification, enrichment procedures, or chromatography. Often optimization of MIPs is required to obtain a reasonable performance. Therefore systematic variation of parameters such as cross-linking-density, type and concentration of monomers, solvent and template molecules is necessary. Recently a combinatorial method was developed for optimization of MIPs by automated small scale synthesis and analysis (Takeuchi et al., 1999). Another combinatorial approach used imprinted polymers as specific binding matrices for the screening of a combinatorial steroid library (Ramstrom et al., 1998).

3.5.2. Colloid crystal templating

Similar to molecular imprinting colloid crystal templating is a transcriptive templating technique. Colloidal crystals exhibit high order on a mesoscopic scale which potentially results in a number of interesting properties, such as extremely high porosities at optimized mechanical strength, the coupling of chemical recognition with photonic band gap sensing, etc. Colloidal crystals are formed in a cooperative crystallization process resulting in maximal packing density of colloidal objects. Usually these colloid crystal templates are very brittle, redispersable and fragile. Using a colloidal crystal as a template with precursors which do not affect the crystalline structure enables replication of the crystal morphology into a durable material. The synthesis is always performed in a two-step procedure, the assembly to a crystalline template morphology followed by swelling and polymerization of precursors. After polymerization the template is removed by extraction, dissolution or calcination, thus resulting in a porous, periodically ordered polymer. The strategy of colloid crystal templating was first established for inorganic materials but works in the same way for organic polymer gels (Velev and Lenhoff, 2000; Xia et al., 2000) as it will be shown in following examples.

Porous polyurethane membranes were formed by templating latex crystals assembled by injection and accumulation between two solid plates (Park and Xia, 1998a,b). After UV-polymerization the polystyrene lattices were removed by selective extraction with toluene. Similarly, colloidal crystals of monodisperse silica particles were used as templates and removed by hydrofluoric acid after UV-polymerization of polyurethanes and poly(acrylate-methacrylate) (Gates et al., 1999).

Colloidal assembly of latex particles towards crystals by pressing microspheres with an average diameter of < 100 nm in the solid state was demonstrated by Johnson et al. (1999). Mechanical properties and the fine structure of the polymers were adjusted by copolymerization of divinylbenzene (DVB) and ethyleneglycol-dimethacrylate (EGDMA). By applying a convective assembly method Jiang et al. prepared free-standing, optically active porous films from a wide



Fig. 5. polyurethane as a template. © 1999 Interscience contact

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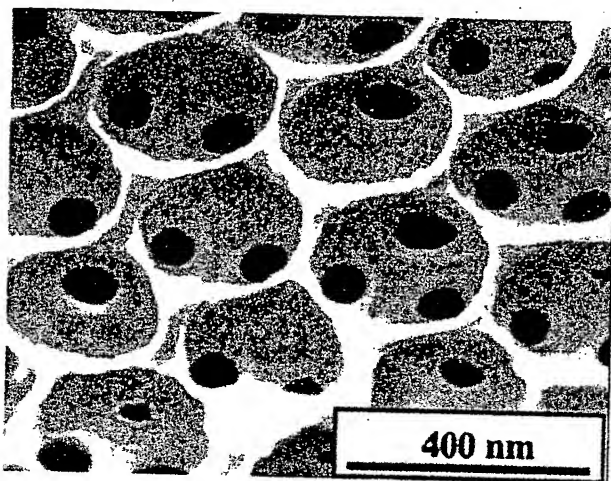


Fig. 5. SEM micrograph of the inner layer of a macroporous polyurethane membrane using a colloidal silica sphere crystal as a template (Reproduced with permission from Jiang et al. © 1999 American Chemical Society) (Jiang et al., 1999). Intercellular pores are formed where silica spheres were in contact with each other during template synthesis.

variety of polymers, such as polystyrene, poly(methyl methacrylate) or flexible polyurethane (Jiang et al., 1999). Again crystals of colloidal silica (artificial opals) were used as templates. The obtained polymers showed a connected pore morphology by opening windows within the templated walls of the pores (Fig. 5).

So far only templating by latex particles has been described in the literature. The use of other crystalline templates with mesoscale order and large channel systems, like protein crystals, is expected to work in a similar fashion. This way more complex morphologies of porous polymers will be realized which combine the advantages of molecular imprinting and the long range order of crystalline materials.

3.5.3. Polymerization from microemulsions and lyotropic liquid crystals

Porous polymers are also obtained by polymerization from ordered surfactant phases or microemulsions. Microemulsions are thermodynamically stable mixtures of oil, water and a surfactant. They form spontaneously and are optically transparent or slightly opaque. If one or both of the subphases contain a monomer, polymerization reactions can be performed confined

by the geometry of the microemulsion, ending up with rather well controlled porous polymer morphologies. Opposite to globular microemulsions with a reverse or direct spherical aggregate structure, sponge phases of bicontinuous microemulsions exhibit continuous water and oil channels interwoven into each other. Monomers located in one or both of these dispersed phases or the use of polymerizable surfactants often lead to continuous, porous polymer gels (Gan and Chew, 1997). Copolymerization of methylmethacrylate, ethyleneglycoldimethacrylate and acrylic acid for instance resulted in open porous polymer gels with a bicontinuous structure on the micrometer scale (Raj et al., 1993). Here and in similar approaches, the obtained polymer gels do not resemble the original microemulsion structure, suggesting a particle nucleation and growth mechanism (Davis et al., 1998; Raj et al., 1995; Schmuhl et al., 1998). This hypothesis was confirmed by investigation of phase transitions occurring during the polymerization of styrene, diisopropenylbenzene and acrylic acid in a bicontinuous microemulsion stabilized by cetyltrimethylammonium bromide (Antonietti and Hentze, 1996). At least four different phase transitions were identified by rheology and polarized light microscopy during the course of reaction. Again the bicontinuous polymer gels obtained were structured on the hundreds of nanometers to micrometer scale and showed a substructure of clustered polymer spheres forming the continuous polymer matrix as characterized by electron microscopy.

Copolymerization of monomers with polymerizable surfactants was performed for a fine tuning of interfacial interactions of the growing polymer matrix and the microemulsion (Puvvada et al., 1994). Even if there is no direct proof for the preservation of the original microemulsion structure with the porous polymer, at least the obtained gels are ordered on the same length scale of the polymerization medium (average pore diameters $\sim 1\text{--}70\text{ nm}$). Polymers prepared that way were applied as size selective membranes (Liu et al., 2000).

Besides low molecular weight surfactants, also less soluble lipids were applied for the formulation of polymerizable bicontinuous microemul-

sions (Puvvada et al., 1994), as well as polymerizable low molecular weight surfactants (surfmers) (Chew et al., 1998; Chieng et al., 1996a; Gan et al., 1995; Li et al., 1996, 1997; Zhang et al., 2000) and polymerizable amphiphilic polymers (macro-surfmers) (Gan et al., 1997; Liu et al., 1997, 2000). Also the chain length of the used surfactants can have a huge impact on the observed polymer morphologies (Chieng et al., 1996b), which again underlines that the mechanism of structure formation differs from a simple casting of the original microemulsion structure. More detailed studies of the mechanism by SAXS, BET measurements and electron microscopy showed the occurrence of phase separations (Burban et al., 1995).

Another type of self-organized templates are liquid crystalline phases, e.g. lyotropic mesophases, block copolymer phases and polyelectrolyte surfactant complexes. They form spontaneously, are thermodynamically stable and combine properties of crystals (long-range order) and liquids (fluidity). Due to their inherent long range order, the morphological complexity of liquid crystalline templated polymer gels is usually higher. Furthermore, they offer the possibility of formation of anisotropic pore morphologies in polymer structures.

Different from micellar solutions or microemulsions, the amphiphile concentration in such recipes is usually very high, i.e. surfactant is the major component of such polymerization reactions. This way thermodynamic complexity is decreased, and it was expected that the stability of the phases against demixing is improved.

Usually, increasing turbidity during the reaction and a detailed investigation of the isolated polymer morphology gives evidence for demixing into a polymer rich and a surfactant rich phase (Antonietti et al., 1999). Hence the lc order observed by scattering techniques after polymerization is most likely due to a subphase containing the lyotropic template, which remains within the demixed polymer gel matrix after polymerization. A detailed investigation of structure formation during polymerization of hydrophilic monomers within hexagonal phases indicated nucleation and growth of a polymer rich subphase within the reaction mixture (Antonietti et al.,

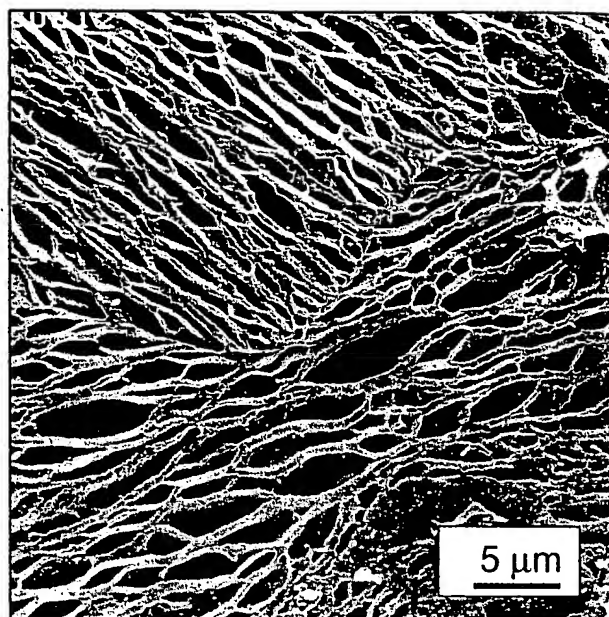


Fig. 6. Scanning electron micrograph of a poly(acryl amide) gel (Antonietti et al., 1998). Directed polymer growth within the anisotropic reaction media resulted in a layer-like polymer morphology (Reproduced by permission from Antonietti et al. © 1998 American Chemical Society).

1998). In a first step polymer particles are formed which separate from the lyotropic phase and assemble towards sheet-like structures directed by the anisotropy of viscosity and transport properties within the single domains of the lyotropic template (Fig. 6). Definition and extension of the single polymer gel layers depend on the degree of cross-linking. Chemical functionalization and mechanical properties can be adjusted by copolymerization with functional comonomers like acrylic acid.

A key for the understanding of these phenomena may be found in the recent observation that already demixing of silicon oils from nematic liquid crystalline phases results in formation of highly ordered polymer arrays, i.e. exactly parallel aligned chains of polymer droplets (Loudet et al., 2000). It was concluded that colloidal ordering is directed by force fields between the defects created by the demixing process, thus resulting in strong order phenomena on the micron scale rather than the nanometer scale of the original liquid crystal.

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mers were also used to create ordered epoxy-networks (Lipic et al., 1998). Selective swelling of one of the block copolymer segments with pre-polymers results in long-range ordered, curable assemblies. After polyaddition of the pre-polymers a resin-block copolymer composite is formed which shows, due to the kinetic hindrance, practically the original structure of the template, i.e. the demixing is confined to the block copolymer aggregates.

Even if direct replicating of lyotropic mesophases is still not realized a huge variety of new and at least as promising possibilities open up like the formation of hierarchical structures in phase separating non-equilibrium reaction mixtures. It must be reminded that the most complex morphologies created by biological systems origin from non-equilibrium systems. Hence phase separation, demixing and non-equilibrium states should not be regarded as a limit of creating highly ordered morphologies, but vice versa they enable the formation of even more complex and versatile morphologies.

3.6. Functionalization of porous polymers

For all physical applications, implementation of a distinct pore structure into the polymer gel is sufficient to obtain the desired properties, e.g. lightweight design or low dielectric constant. For chemical applications, another (practically orthogonal) parameter is key, namely the polarity and the chemical functionality of the very large surface of the pore system. This surface structure determines wettability or hydrophilicity/hydrophobicity, which is important for solvent uptake and interaction with the environment. Distinct chemical groups are important for selective binding or the catalytic properties.

It is one of the very big advantages of porous polymers (as compared with porous inorganics) that this functionalization is comparably easy and sometimes just performed by copolymerization with an appropriate functional comonomer. In many cases, addition of such a monomer, however, influences the mixing thermodynamics and steps into the process of pore formation, as outlined above. In this case the final polymer gel can

be functionalized by polymer reactions in a second step. Another attractive possibility is functionalization by physical means, i.e. by a precipitation reaction onto or a coating of the polymer structure. Examples for these different procedures are given in the following paragraphs.

Functionalization of highly cross-linked porous poly(styrene-co-divinylbenzene) beads with *tert*-butyl groups resulted in a material suitable for very fast separation of pollutants (Klampfl and Spanos, 1995). All 11 priority pollutant phenols classified by the US Environmental Protection Agency (EPA) could be separated using a HPLC column packed with the functionalized resin.

By functionalization of porous resin particles resin-loaded membranes were produced which can be used for solid-phase extraction (SPE) (Fritz et al., 1995). Using such efficient particles for separation could replace liquid extraction procedures which require large amounts of organic solvents. For SPE, the resin particles have been partly sulfonated to increase the efficiency of the extraction of polar organic compounds.

A continuous monolith of porous poly(glycidyl methacrylate-co-ethylene dimethacrylate) has been prepared by a free radical polymerization within the confinement of a 16-mm glass column (Svec and Frechet, 1995a). The epoxide groups of the polymer have been modified in situ by their reaction with diethylamine to afford the ionizable weak base 1-*N,N*-diethylamino-2-hydroxypropyl functionalities that are required for the ion-exchange chromatographic mode. The bimodal pore size distribution curve typical for other molded monolith separation media also prevail for this system. The column has been used successfully for the chromatographic separation of a mixture of standard proteins and yeast enzymes. The column exhibits a dynamic capacity that exceeds 420 mg of bovine serum albumin at a flow velocity of 60 cm h⁻¹.

A copolymer comprised of glycidyl methacrylate and *N,N*-methylenebis(acrylamide) has been described that can be used to coat solvent-impregnated resins (SIRs). Vinyl groups on the surface of a cross-linked macroporous support were used to anchor the polar copolymer to the surface of the bead. The coated SIR containing di(2-eth-

ylhexyl)phosphoric acid maintained a high level of metal ion complexation [96% Cu(II)] over five cycles while the uncoated SIR dropped from 93% to 11% Cu(II) complexed in three cycles (Alexandratos and Ripperger, 1998).

In order to prepare phosphoric acid resins (RGPs) with large cation exchange capacities, the effects of porosity and cross-linking of the precursor poly(glycidyl methacrylate-co-divinylbenzene) beads on their functionalization with phosphoric acid were studied (Jyo et al., 1997). It was clarified that porosity of the precursors plays an important role in the functionalization. Highly porous precursors were functionalized with high efficiency; for example, even the precursors containing 10 mol.% of divinylbenzene resulted in RGPs having cation exchange capacities as large as 6–7 mEq g⁻¹, as long as the BET specific surface areas of the precursors were greater than approximately 30 m² g⁻¹. The selectivity study has revealed that RGP exhibits the characteristic metal ion selectivity of phosphoric acid derivatives. Lithium ions were adsorbed in preference to sodium and potassium ions, and hard Lewis acid cations, such as uranyl, ferric, and aluminum ions, are adsorbed even from strongly acidic media (1 < pH < 2). Among the common divalent metal ions, in addition, the resin exhibits the highest selectivity toward lead ions.

PolyHIPEs (s. 4.9.4.3) bearing pendant vinyl functionalities were prepared by chemical post-functionalization (Mercier et al., 2000). The large pores and large channels of polyHIPEs allow liquids and solvents to be driven through the molded monolith at very low pressure. In the presence of a free-radical initiator, compounds such as HBr and thiols underwent an anti-Markovnikov addition to the residual vinyl groups. Other reactions such as hydroboration with BH₃ followed by hydrolysis with H₂O₂ have also been investigated. A comparison between two ways of functionalization was made: a 'batch' method, corresponding to diffusion of reactants through the pores of small cubes of polymers, and a 'continuous flow' method, forcing reactants to flow through the interconnected porous structure by applied low pressure.

A number of electrophilic aromatic substitution

reactions, namely sulfonation, nitration and bromination were performed for the functionalization of highly porous poly(styrene/DVB) polyHIPE monoliths (Cameron et al., 1996b). Mild, hydrophobic reagents and homogeneous reaction conditions were sought in an effort to achieve uniform chemical modification at a reasonable degree of substitution throughout the large porous polymer gel. Thus, sulfonation was carried out with lauroyl sulfate in cyclohexane, nitration with tetrabutylammonium nitrate-trifluoroacetic anhydride (TBAN-TFAA) in dichloromethane and bromination with bromine-stannic chloride in dichloromethane. The differences in extent of each reaction was explained by consideration of such factors as the nature of the solvent, polarity of the reagents and compatibility between the reagents and the polymer matrix throughout the reactions.

Disks obtained by polymerization of high internal phase emulsions had completely open pore structures and were used as a carrier material for the immobilization of 10-ethyl-isoalloxazine ('flavin') (Schoo et al., 1992). Three methods for immobilization are described: (1) chemical modification of chloromethylated polyHIPE with flavin and triethylamine; (2) deposition of a polyelectrolyte complex of flavin-containing polycations and poly(sodium styrenesulfonate) onto the (internal) surface of a polyHIPE; and (3) complexation of flavin-containing polycations on the (internal) surface of a sulfonated polyHIPE. The catalytic activity per flavin moiety in the continuous aerobic oxidation of 1-benzyl-1,4-dihydronicotina-mide depends on the method of immobilization and on the loading with the catalytic moieties, factors influencing the accessibility and distribution of catalytic sites over the pore surface and the matrix of the polyHIPE. Optimum activity was found in the case of method 3. All methods resulted in high stability of the immobilized catalyst in continuous reactions. The linear polycations immobilized by methods 2 and 3 can be decomplexed completely after the continuous reaction, using a ternary solvent, and analyzed by homogeneous spectroscopic techniques, providing a powerful tool in the study of the fate of catalysts in continuous processes.

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4. Applications of porous polymers and resins

Porous polymer gels or materials can possess outstanding and unusual properties in nature as well as in technology. Typical examples for biological systems are bones. Here extreme toughness is gained by combining typical properties of a porous organic structure (ductility, elasticity, lightweight design) with those of inorganic minerals (hardness). This way a high performance ceramic material is obtained.

Also for separation applications such as selective membranes, chromatographic materials, or in gel electrophoresis (Righetti, 1995), pore size and pore connectivity are of central importance. Porous membranes templated within bicontinuous microemulsions were used as ultrafiltration membranes (Li et al., 1996, 1997; Liu et al., 2000) or for gel electrophoresis. In addition, porous polymer gels offer the opportunity to adjust also molecular interactions, for instance for stereoselective chromatography and enantio-selective catalysis. Therefore the combination of a hierarchical porosity with molecular imprinting seems to be most suitable for these applications (Sellergren, 2000).

The effect of the porous structure of 2-mm-thick diethylamine functionalized monolithic polymethacrylate discs on their chromatographic behavior in ion-exchange mode was studied (Tenikov et al., 1998). Discs with small pores did not perform well because they exhibited high back pressure and substantial peak broadening. Discs characterized with pores larger than 1000 nm did not provide good separations either because the time required for some protein molecules to traverse the length across the pore to reach the wall for adsorption/desorption process that is essential for separation may be longer than their residence time within the pellet. The optimum pore size was centered at approximately 700 nm. Excellent separations have been achieved with these discs even at very steep gradients and high flow-rates which allow to shorten the separation times substantially.

Templated porous polymers do not only find application in analytics, but also as mesostructured reaction media. The use of porous polymer

gels made from bicontinuous microemulsions for the synthesis of organic–inorganic nano-hybrids was demonstrated by polymer directed mineralization of iron oxide within a bicontinuous polystyrene-co-polyacrylic acid gel (Breulmann et al., 1998). Here, a nanocomposite consisting of a porous polymer gel functionalized by homogeneously distributed, superparamagnetic magnetite particles of approximately 15 nm diameter was obtained. Interestingly, the observed morphology is reminiscent of the structure of a mollusk tooth which consists of magnetite colloids of comparable sizes within a protein–polysaccharide matrix.

Potential pharmaceutical applications of templated porous polymer gels are controlled drug delivery devices with delivery characteristics depending on the pore morphology. Several reviews discussing the potential of molecularly imprinted polymers (MIPs) for applications in chromatography and sensors have recently been published (Mayes and Mosbach, 1997; Piletsky et al., 1999). Also pharmaceutical applications, such as controlled release, drug monitoring devices and biological receptor mimetics are in discussion (Allender et al., 2000). For instance highly selective composite membranes for pharmaceutical or biomedical separations were obtained by photopolymerization of a molecularly-imprinted polymer film across the surface of a microporous support to form a new type of ultrathin film composite membrane. Composite membranes based on polymers that were imprinted with bronchodilator theophylline were prepared. Efficient rates and selectivities of theophylline transport across these ultrathin film composites were observed (Hong et al., 1998). Even if most of the potential applications aim at separation techniques, MIPs are also useful materials for other processes, such as catalysis (Alexandratos and Ripperger, 1998).

Anisotropic materials with selective binding properties may have potential as active components in optical sensors. A novel route for the synthesis of such materials was recently described, and the validity of the concept has been demonstrated. Free radical polymerization of 2-ethyl-2-(hydroxymethyl)-1,3-propanediol trimethacrylate (TRIM) in the presence of a tem-

plate molecule [4,4'-bis(dimethylamino)benzophenone (Michler's ketone)] and the binding sites [2-(acrylamido)-2-methylpropanesulfonic acid (AMPSA) or methacrylic acid (MAA)] has led to molecularly imprinted polymer monoliths. Upon irradiation using linearly polarized light, the template molecules reacted with the polymer networks to form transparent and anisotropic polymer monoliths. The origin of the dichroism is discussed and is believed to result from the incorporation of reactive template molecular species (radicals) into the polymer network. The imprinting of the polymer gels was confirmed using competitive binding studies. The polymers show size and shape selectivity but are not always in favor of the template molecule (Steinke et al., 1996).

Engineering new bone tissue including incorporated cells and a synthetic extracellular matrix (scaffolding) represents a new approach for the regeneration of mineralized tissues and competes with the transplantation of bone (autografts or allografts) (Ma et al., 2001). Highly porous poly(L-lactic acid) (PLLA) and PLLA/hydroxyapatite (HAP) composite scaffolds were prepared by thermally induced phase separation. Osteoblastic cells were seeded into the porous polymer and cultured in vitro. In the pure PLLA scaffolds, the osteoblasts attached primarily to the outer surface of the polymer, which was not desired. When loaded with hydroxyapatite, the osteoblasts, however, penetrated deeply into the PLLA/HAP scaffolds and were uniformly distributed. The percentage of osteoblast survival in the PLLA/HAP scaffolds was superior to that of pure PLLA scaffolds. The osteoblasts proliferated in both types of the scaffolds, but the cell number was always higher in the PLLA/HAP composite scaffolds after 6 weeks of in vitro cultivation. Bone-specific markers (mRNAs encoding bone sialoprotein and osteocalcin) were expressed more abundantly in the PLLA/HAP composite scaffolds than in the PLLA scaffolds.

The ability of neurons to survive and attach onto macroporous polylactide (PLA) foams was assessed in vitro (Maquet et al., 2000). The foams were fabricated by thermally induced polymer-solvent phase separation. Two types of pore structures, namely oriented or interconnected pores,

were produced, depending on the mechanism of phase separation, which in turn can be predicted by the thermodynamics of the polymer-solvent pair. Coating of the porous foams with polyvinyl-alcohol (PVA) considerably improved the wettability of the foams and allowed the cells to culture. The in vitro biocompatibility of the PVA-coated supports was demonstrated by measuring cell viability and neuritogenesis. Microscopic observations of the cells seeded onto the polymer foams showed that the interconnected pore networks were more favorable to cell attachment than the anisotropic pore structures. The capacity of highly oriented foams to support in vivo peripheral nerve regeneration was studied in rats. A sciatic nerve gap of 5 mm length was bridged with a polymer implant which possesses macrotubes of 100 μm diameter. Four weeks after operation, the polymer implant was still present. It was well integrated and had restored the anatomic continuity. An abundant cell migration was observed at the outer surface of the polymer implant, but not within the macrotubes. This dense cellular microenvironment was found to be favorable for axogenesis.

PolyHIPE monoliths were used to produce a polyamide-impregnated composite support for the use in solid phase synthesis of peptides. The composite showed good pressure resistance and allowed high yields of pure peptides to be achieved (Sherrington, 1993). PolyHIPEs with very high surface areas (up to $350\text{ m}^2\text{ g}^{-1}$) were prepared by applying the methodologies of porous particulate resin production to achieve highly porous walls in the polyHIPE structures.

Homogeneous, highly porous, low density polyHIPEs were produced by polymerization of a high internal phase emulsion (Wakeman et al., 1998) and modified by sulfonation. By incorporating Na^+ ions onto the sulfonated polyHIPE surfaces a special form of ion exchange resin capable of effective exchange with metal ions in solution was produced. Isotherms were measured for samples of polyHIPE with differing degrees of sulfonation and compared with data obtained from commercially available ion exchange resins. The capacity of the polyHIPE resin increased with the degree of sulfonation. In single-pass dynamic adsorption

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tests the new material showed even better column performance than the commercial resins.

Cross-flow filtration modules made from Poly-HIPEs have been tested using a computer-controlled filtration unit to investigate the effects of membrane skin porosity and surface modification on their filtration performance (Bhumgara, 1995).

This list of examples is far from being complete and is just meant for illustration. Although the main application focus for porous polymers is so far still on chromatography and membrane technologies, we expect future work to be possibly more concentrated on the chemistry directed or enabled by the confined geometries of these restricted environments (nano- and microreactor technologies).

5. Conclusion and outlook

From the very beginning of polymer gel chemistry to the high tech porous materials of today a continuous development towards more complex morphologies and more versatile methods of functionalization took place. With increasing complexity and material performance, porous polymers and resins became also more important, not only for fundamental research, but also for technical applications. Classical synthesis routes towards porous polymers and resins, such as induced phase separation or the use of gaseous and liquid porogenes, were continually optimized to obtain high performance polymers for certain applications, for instance electrophoresis gels or supports for solid-phase chemistry. In the last 10 years also supercritical media, such as scCO_2 , were added to the list of porogenes, providing a number of advantages. Recently, concepts of template synthesis and the employment of self-organizing templates have also become increasingly important for the synthesis of porous materials. Depending on the type of template and the employed self-organization mechanism, isotropic, anisotropic, or hierarchical gel structures can be obtained. Current research in the field of porous polymer synthesis is mostly focused to design new gel structures with new functionalities by under-

standing the general principles of structuring organic matter.

Applications of porous polymers are as diverse as molecular recognition, high throughput analysis and separation media, or solid supports for high flow throughput synthesizers. New tools of porogenesis will inspire researchers and engineers to develop new techniques which will influence science as well as daily life, for instance for biotechnological and pharmaceutical applications. Finally, tissue engineering or design of bioconvertible implants and transplants relies on biodegradable, chemically functionalized porous polymer scaffolds which bridge between the worlds of synthetic polymers and the biological world.

The integration of natural principles like template synthesis and self-organization into the strategy of chemical synthesis opens up the huge potential for structuring materials over all length scales starting from molecules to structures on the nanometer and micrometer length scale. These techniques are especially powerful for the structuring of porous polymers and resins. It is the hope and promise that mimicking of these biological principles enables the transfer of the superior properties and property combinations of biological materials towards large scale synthetic materials, as well as analytical and synthetic procedures.

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